

Phase II study of temozolomide, thalidomide, and celecoxib for newly diagnosed glioblastoma in adults

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We conducted a phase II study of the combination of temozolomide and angiogenesis inhibitors for treating adult patients with newly diagnosed glioblastoma. Patients who had stable disease following standard radiation therapy received temozolomide for 5 days in 28-day cycles, in combination with daily thalidomide and celecoxib. Patients were treated until tumor progression or development of unacceptable toxicity. Four-month progression-free survival (PFS) from study enrollment was the primary end point, and overall survival (OS) was the secondary end point. In addition, we sought to correlate response with O⁶-methylguanine-DNA methyltransferase promoter methylation status and serum levels of angiogenic peptides. Fifty patients with glioblastoma were enrolled (18 women, 32 men). Median age

was 54 years (range, 29–78) and median KPS score was 90 (range, 70–100). From study enrollment, median PFS was 5.9 months (95% confidence interval [CI]: 4.2–8.0) and 4-month PFS was 63% (95% CI: 46%–75%). Median OS was 12.6 months (95% CI: 8.5–16.4) and 1-year OS was 47%. Of the 47 patients evaluable for best response, none had a complete response, five (11%) had partial response, four (9%) had minor response, 22 (47%) had stable disease, and 16 (34%) had progressive disease. Analysis of serial serum samples obtained from 47 patients for four angiogenic peptides failed to show a significant correlation with response or survival for three of the peptides; higher vascular endothelial growth factor levels showed a trend toward correlation with decreased OS ($p = 0.07$) and PFS ($p = 0.09$). The addition of celecoxib and thalidomide to adjuvant temozolomide was well tolerated but did not meet the primary end point of improvement of 4-month PFS from study enrollment. *Neuro-Oncology* 10, 300–308, 2008 (Posted to *Neuro-Oncology* [serial online], Doc. D06-00231, April 10, 2008. URL <http://neuro-oncology.dukejournals.org>; DOI: 10.1215/15228517-2008-005)

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Despite optimal treatment with surgery, radiation, and chemotherapy, the prognosis for patients with malignant gliomas remains poor. First-line chemotherapy with temozolomide has been shown to improve survival when given with radiotherapy.¹ Because gliomas are highly angiogenic^{2–6} and the more malignant tumors show evidence of greater angiogenic activity in the form of high microvessel density and neovascularization, we investigated whether the addition of angiogenic inhibitors (thalidomide and celecoxib) in combination with cytotoxic chemotherapy (temozolomide) would be more efficacious.

Malignant gliomas may induce angiogenesis by secreting factors with angiogenic properties.^{7–9} Examples of these factors include acidic fibroblast growth factor and basic fibroblast growth factor (bFGF), angiogenin, vascular endothelial growth factor (VEGF), platelet-derived growth factor, interleukin-8, hepatocyte growth factor, and tumor necrosis factor- α . Experimental work in animals^{10–14} has demonstrated promising reductions in tumor growth using approaches that inhibit angiogenesis. Although blockade of angiogenic factors holds promise as a strategy for patients with malignant glioma,¹⁵ effective application of such a strategy has been constrained until recently by the limited availability of potent inhibitors of angiogenesis.

In this study, we evaluated the combination of temozolomide with two oral inhibitors of angiogenesis, thalidomide and celecoxib. Thalidomide has shown modest antiglioma activity as a single agent^{16,17} and in combination with carmustine,¹⁸ possibly by inhibition of bFGF.¹⁶ Celecoxib is a selective cyclooxygenase-2 (COX-2) inhibitor that has recently been shown to have antiangiogenic activity, in addition to its better-known anti-inflammatory actions. Celecoxib may also inhibit bFGF and VEGF.^{19,20} COX-2 is highly expressed in glioma cells and tumor vasculature,²¹ and inhibition of COX-2 has shown efficacy in preclinical rat models of solid tumors.²² Early-phase clinical studies of celecoxib have been promising in some solid tumors.^{23–26}

Preclinical data have shown promising results using combinations of cytotoxic and anti-angiogenic therapies in animal models of solid tumors.^{27,28} Recently, a number of clinical studies of antiangiogenic agents such as the VEGF monoclonal antibody bevacizumab (Avastin) have shown significant activity in a number of cancers when combined with chemotherapy.^{29–31}

Because of the angiogenic nature of human malignant gliomas, the promising early results of antiangiogenic chemotherapy regimens in other human cancers, and the safety, convenience, and tolerability of the current regimen, we conducted a phase II trial to determine the tolerability and efficacy of a three-drug regimen (temozolomide, thalidomide, celecoxib) in patients with newly diagnosed glioblastoma in the postradiation setting. All three drugs were administered orally and have been well tolerated in this population, offering a significant quality of life advantage for patients. The potential synergis-

tic effects of temozolomide, thalidomide, and celecoxib have not been explored to date in adults with glioma.

Materials and Methods

Objectives

The primary objective of this study was to determine the efficacy of oral administration of temozolomide, thalidomide, and celecoxib in patients with newly diagnosed glioblastoma or gliosarcoma in the postradiation setting. Four-month progression-free survival (PFS) was the primary end point, and overall survival (OS) was the secondary end point. We also evaluated the safety/toxicity of this three-drug combination. In addition, we sought to correlate response with methylation status of the MGMT (*O*⁶-methylguanine-DNA methyltransferase) promoter³² and with levels of serum angiogenic peptides.³³

Patient Eligibility

Between May 2001 and October 2003, eligible adult patients were enrolled after they gave written informed consent. The institutional review boards of the Dana-Farber/Harvard Cancer Center and the University of Virginia approved this protocol before enrollment (DFCI protocol 00-302). Patients with histologically proven supratentorial glioblastoma or gliosarcoma who had stable disease following completion of standard external beam radiotherapy were eligible for this study. Patients had to be registered within 5 weeks of completing radiotherapy. They could not have received prior chemotherapy, Gliadel wafers, or treatment with thalidomide, and they were required to be on a stable dose of corticosteroids at time of enrollment. In addition, they had to meet the following criteria: age ≥ 18 years; life expectancy > 4 months; KPS score ≥ 60 ; adequate hematological function (absolute neutrophil count [ANC] $\geq 1,500/\text{mm}^3$, platelets $\geq 100,000/\text{mm}^3$); adequate liver function (serum glutamic pyruvic transaminase and alkaline phosphatase < 2.5 times greater than normal, bilirubin < 1.5 mg%); adequate renal function (blood urea nitrogen or creatinine < 1.5 times greater than upper limits of institutional normal). Patients were excluded if they were pregnant or nursing or had greater than National Cancer Institute Common Toxicity Criteria (NCI CTC) version 2.0 grade 1 peripheral neuropathy, serious concurrent medical illness, history of other cancers and received therapy within the past 3 years, refusal to follow the required birth control measures during and for 4 weeks after treatment with thalidomide, or concurrent use of other investigational agents.

Treatment Regimen

Patients were treated with temozolomide given initially at 150 mg/m²/day for 5 consecutive days for the first 28 days. Barring significant toxicities, the dose of temozolomide in subsequent cycles was increased to 200 mg/m²/

day for 5 days every 28 days. Thalidomide and celecoxib were given daily, continuously throughout the cycle. Thalidomide was begun at a dose of 200 mg orally at bedtime and escalated by 50 mg every 1–2 weeks as tolerated to a maximum of 1,200 mg daily. Celecoxib was begun at a dose of 200 mg twice daily after meals and increased to 400 mg twice daily if tolerated within 4 days. Therapy was continued until evidence of progression or unacceptable toxicity.

Dose Modifications and Patient Follow-up

Patients were closely monitored throughout therapy for drug-related toxicity, and all adverse events were recorded and graded according to the NCI CTC version 2.0. Pregnancy testing in women of child-bearing potential was performed weekly during the first 28-day cycle and then on day 1 of every subsequent cycle in women with regular menstrual cycles, and every 2 weeks in those with irregular menstrual cycles. All patients were required to participate in the mandatory System for Thalidomide Education and Prescription Safety (S.T.E.P.S.) program. Physical and neurological examinations were performed every 4 weeks. Hematological testing was performed weekly for the first two cycles and then on days 1 and 21 of every subsequent cycle. Study drugs were held if a patient experienced drug-related grade 3 nonhematological toxicity, grade 3 thrombocytopenia, or grade 4 neutropenia and anemia. Patients, in conjunction with treating physicians, could reduce doses of medications if toxicity was seen at higher doses. The dose of temozolomide was titrated in subsequent cycles based on the patients' tolerance to the therapy to lowest level of 100 mg/m²/day for 5 days. Thalidomide and celecoxib were reduced to prior dose level for 1 week, with decisions to further reduce dosage for 1 week or restart dose escalation (if toxicity had resolved) made by the patients in concert with treating physicians. A new 4-week cycle could begin when there was adequate hematological recovery (ANC \geq 1,500/mm³ and platelet count \geq 100,000/mm³) and any nonhematological, symptomatic toxicities were no more severe than grade 2.

Imaging and Response Assessment

MRI of the brain was performed every two cycles (8 weeks). Axial and coronal T1 pre- and postgadolinium images, among others, were obtained and used for this study. Responses were determined using modified Macdonald criteria³⁴: complete response—complete disappearance of tumor; partial response—at least 50% decrease in the sum of products of the two largest perpendicular diameters of all measurable lesions; minor response—between 0 and less than 50% decrease in the sum of products of the two largest perpendicular diameters of all measurable lesions; progressive disease—at least 25% increase in the sum of products of the two largest perpendicular diameters of all measurable lesions; stable disease—neither minor response, partial response, nor progressive disease. The criteria were applied in the

setting when patients were on a stable dose of steroids and did not experience clinical deterioration other than that attributable to progressive tumor burden (e.g., systemic or metabolic disturbances). Responses (complete, partial, minor) had to be sustained on two successive scans taken at least 4 weeks apart compared with the best response scan.

Angiogenic Peptide Measurements

Antiangiogenic activity was measured by assays of angiogenic peptides as a biomarker of tumor response to the drug regimen.^{33,35} VEGF, bFGF, endostatin, and thrombospondin-1 (TSP-1) levels were evaluated from batched samples of serum (when available and with consent) using commercially available ELISA kits (VEGF and bFGF kits were obtained from R&D Systems, Minneapolis, MN, USA; endostatin and thrombospondin kits were obtained from Cytimmune Sciences, College Park, MD, USA) in accordance with the manufacturers' recommended methodology. Patients were requested to provide blood samples before therapy and approximately every fourth week while receiving treatment.

DNA Extraction and Methylation-Specific Polymerase Chain Reaction

Genomic DNA was isolated from 3 \times 10 μ m paraffin tissue sections of glioblastoma tissue containing at least 70% tumor (QIAamp DNA Mini Kit, Qiagen, Valencia, CA, USA). DNA methylation patterns in the CpG island of the MGMT gene (Genbank accession no. AL355531 nt 46931–47011) were determined by chemical (bisulfite) modification of unmethylated, but not methylated, cytosines to uracil (CpGenome Fast DNA Modification Kit, Chemicon brand, Millipore, Billerica, MA, USA). The bisulfite-treated DNA was precipitated, rehydrated, and amplified by a methylation-specific polymerase chain reaction using primers specific for either the methylated or the modified unmethylated DNA 36: methylated forward primer, FAM-5-TTTC-GACGTCGTAGGTTTTTCGC-3, methylated reverse primer, 5-GCACTCTTCCGAAAACGAAACG-3; unmethylated forward primer, 5-GCACTCTTCCGAAAACGAAACG-3-FAM, unmethylated reverse primer, 5-AACTCCACACTCTTCCAAAAACAAAACA-3. PCR amplification with Taq-Gold (Applied Biosystems, Foster City, CA, USA) was performed as follows: 95°C for 10 min, then denature at 95°C for 45 s, anneal at 60°C for 45 s, extend at 72°C for 60 s for 40 cycles, followed by a 10-min final extension at 72°C. PCR products were analyzed in duplicate parallel runs by capillary gel electrophoresis (ABI 3130xl, Applied Biosystems) with an expected product of 93 bp for methylated DNA and 80 bp for unmethylated DNA. Bisulfite-treated DNA from normal placenta and SS I methyltransferase (New England Biolabs, Ipswich, MA, USA)-treated placenta DNA were used as negative and positive controls, respectively. Controls without DNA were performed for each set of PCRs. The sensitivity of the assay based on DNA dilution studies is at least 1:1,000.

Statistical Methods

The primary end point of this phase II study was 4-month PFS in patients with newly diagnosed glioblastoma treated with temozolomide, thalidomide, and celecoxib after radiation. This end point was used because it allowed the results of this study to be compared with those of a similar study of patients with newly diagnosed malignant gliomas, including a majority of patients with glioblastomas, who were enrolled and treated with carmustine or diaziquone (AZQ) following completion of radiation therapy.³⁷ In that study, the median time from start of therapy (study enrollment) to progression for glioblastoma patients was 4 months. In the present study, improvement of median PFS to 8 months would render the combination therapy worth further investigation. Assuming an exponential underlying distribution, 8-month median PFS is equivalent to about a 70% PFS rate at 4 months. We planned to enroll 55 patients, assuming 50 of them to be eligible. This provided 86% power with a one-sided significance level of 0.05. The secondary end point was OS (from study enrollment to death). We also calculated PFS and OS values from date of diagnosis to allow comparison to other studies in the literature.

PFS and OS were estimated using the Kaplan-Meier method. Angiogenic peptide analyses included the assessment of differences between the response categories, in baseline values and the change from baseline to 2-month postenrollment values (which was obtained within 10 days of response date), in its absolute raw form and in log- and percentage-adjusted forms from baseline value. We used the Wilcoxon rank-sum test to determine the significance of these differences. The Cox proportional hazard model was used to determine the effect of angiogenic peptide levels on OS and PFS. Due to the limited sample sizes, the effect of MGMT status on outcomes could not be assessed. All analyses were performed using SAS statistical software (version 8.0, SAS Institute, Inc., Cary, NC, USA).

Results

Population Characteristics

Fifty patients (32 men and 18 women) were enrolled (Table 1). One patient never started treatment after signing informed consent and was lost to follow-up. Ages ranged from 29 to 78 years, with a median age of 54 years. All 50 patients had glioblastoma and had stable disease on MRI at time of enrollment. The median KPS score was 90 (range, 70–100). At the time of enrollment 26 patients (52%) were receiving glucocorticoids, 34 (68%) were receiving enzyme-inducing antiepileptic drugs (EIAEDs), and 16 (32%) were receiving non-EIAEDs. The median follow-up time was 12 months. This was a relatively unselected group of patients, and no patients had a true gross total resection.

Response and Survival

Of the 50 patients, 34 went off study due to progressive disease. The remaining 16 patients, who went off study for other reasons (6 for unacceptable toxicity, 6 on withdrawal of consent, 4 for other reasons), were censored at their off-study date. At the time of analysis, 43 (86%) patients had died. From study enrollment, the median PFS was 5.9 months (95% confidence interval [CI]: 4.2–8.0); the 4-month PFS was 63% (95% CI: 46%–75%), and the 6-month PFS was 40%. The median OS was 12.6 months (95% CI: 8.5–16.4), and the 1-year OS was 47% (95% CI: 33%–60%) (Fig. 1). From date of diagnosis, the median PFS was 9.3 months (95% CI: 8.2–10.9), and the 6-month PFS was 78% (95% CI: 62%–88%). The median OS was 16.1 months (95% CI: 11.8–19.3); the 1-year OS was 58% (95% CI: 43%–71%), and the 2-year OS was 21% (95% CI: 11%–34%). Compared with the postradiation data of Schold et al.,³⁷ our data above for poststudy enrollment showed a 4-month PFS of 63% (the lower bound of the one-sided 95% CI was 43%), which did not meet our requirement for rejecting the null hypothesis, suggesting that there was no evidence for improved efficacy. However, we acknowledge that the de facto power was lower due to eight censored events prior to 4 months (censored events at months 0.1, 0.6, 0.6, 0.7, 1.1, 1.9, 1.9, 2.1, 4.1, 5.6, 6.8, 8.3, 11.3, 11.6, 17.9, and 18.4).

Response could not be evaluated in three patients owing to withdrawal of consent. Of the 47 patients evaluable for best response, no patients (0%) had a complete response, 5 (11%) had partial response, 4 (9%) had minor response, 22 (47%) had stable disease, and 16 (34%) had progressive disease.

Angiogenic Peptide Measurements and Correlation with Response and Survival

We sought in vitro correlates predictive of tumor response to the drug regimen by measuring serum angiogenic peptides during the course of treatment. Serum levels of VEGF, bFGF, endostatin, and TSP-1 were measured in patients who consented to the biological analysis. Of the 50 patients, 47 had at least two samples and 25 had three or more samples available for serum angiogenic peptide testing. Table 2 shows the descriptive statistics

Table 1. Characteristics of the 50 patients

Patient Characteristic	n (%)
Median age, years	54
Age range, years	29–78
Male:female ratio	32:18
Median KPS score	90
Anticonvulsants	
EIAED	34 (68)
Non-EIAED	16 (32)
Glioblastoma	50 (100)

Abbreviation: EIAED, enzyme-inducing antiepileptic drug.

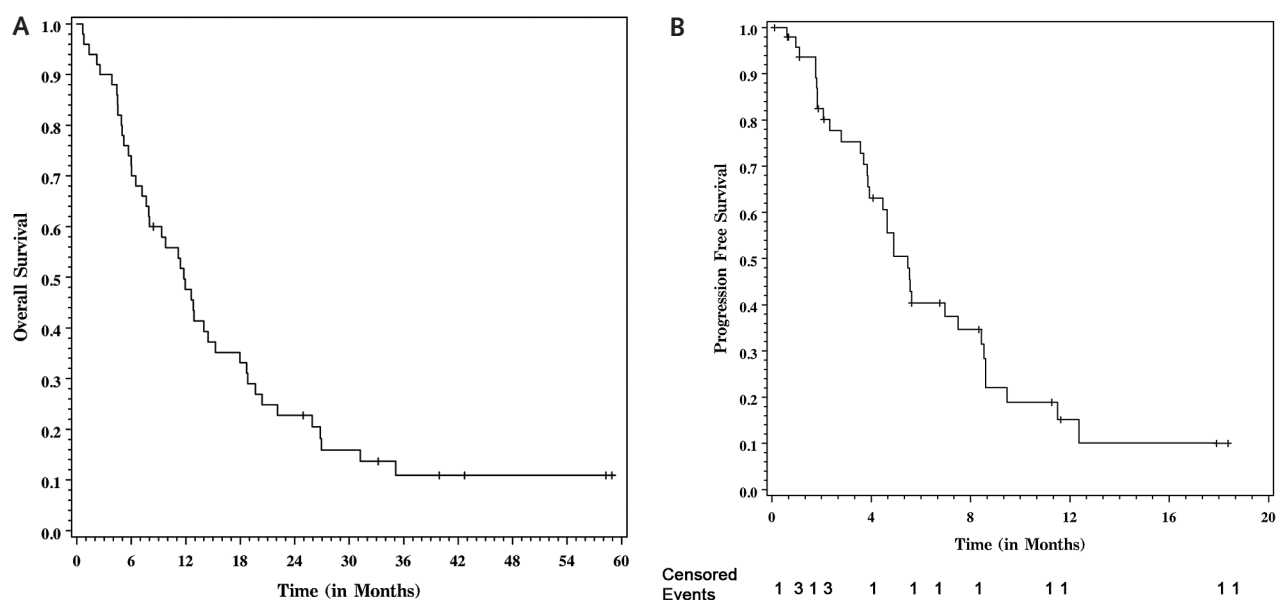


Fig. 1. Kaplan-Meier overall survival (A) and progression-free survival (B) estimates for the entire study population from date of study entry. Hash marks denote censored observations, and the number of censored events is noted below the graph at each time point.

for levels of VEGF, bFGF, endostatin, and TSP-1. We did not find any statistically significant differences between “responders” (partial response + minor response + stable disease) and “nonresponders” (progressive disease) in serum peptide levels (baseline difference from baseline at 2 months and percentage change from baseline at 2 months) of bFGF, VEGF, endostatin, and TSP-1 (i.e., all $p > 0.1$). Using the Cox proportional hazard model, we found that none of the peptides (baseline, difference from baseline at 2 months, and percentage change from baseline at 2 months) affected survival or PFS. When we used the logged version, higher VEGF (i.e., log baseline VEGF) levels showed a trend toward decreased survival and PFS (p -values of 0.07 and 0.09, and hazard ratios 1.39 and 1.47, respectively). Because we were comparing angiogenic peptide values at only two observation points, the possibility remains that there may be more temporally limited correlates of disease activity and drug response that were not detected with our method.

MGMT and Survival

We obtained DNA from paraffin-embedded sections for a retrospective analysis of MGMT promoter methylation status. In initial quality control studies, it appeared that older specimens had poor quality DNA for testing, since after approximately 1 year very few cases were methylated, suggesting that testing on archival paraffin is not reliable and gives false negatives. One of our sites, for example, had only nonmethylated MGMT promoter. We were able to obtain paraffin-embedded blocks from 10 of the 50 patients. Of these, only nine had quality DNA for MGMT promoter methylation and of these only one was methylated. Thus, we had insufficient sample size (power) to correlate MGMT status with response or survival.

Toxicity of Regimen

Therapy was reasonably well tolerated in this patient cohort. The median number of cycles was four (range, 0–20 cycles). Toxicity was monitored and graded (2–4) based on the NCI CTC version 2.0 throughout the trial. There was one treatment-related death due to neutropenia and fever. Although mostly transient grade 1 and 2 toxicities were observed, six patients (12%) discontinued therapy secondary to symptoms possibly or probably related to the treatment protocol after a median of 1.4 cycles. Of these six patients, two had grade 3 leukopenia or neutropenia, two had grade 4 leukopenia or neutropenia, one had a grade 3 rash, and one had grade 4 somnolence. Grade 3–5 toxicities associated with treatment are listed in Table 3. Constipation and fatigue were most common but usually mild. Four venous thromboembolic events were noted in this study.

Discussion

Malignant gliomas are highly resistant to standard chemotherapies. There is increasing evidence that angiogenesis inhibitors, by selectively inhibiting tumor vasculature, may have synergistic activity with cytotoxic agents and would be of tremendous benefit for patients with malignant gliomas where options are limited.^{29–31,38}

Preclinical data suggest strong synergism between temozolomide and thalidomide.²⁷ In this phase II study, we evaluated the therapeutic efficacy of the conventional chemotherapeutic agent temozolomide with two oral angiogenesis inhibitors, thalidomide and celecoxib, in patients with newly diagnosed glioblastomas. Several ongoing human studies are using this approach for a variety of cancers.

Previous phase II trials of thalidomide for recurrent

Table 2. Descriptive statistics of antiangiogenic peptides ($n = 47$)

Variable ^a	<i>n</i>	Mean	SD	Minimum	Lower Quartile	Median	Upper Quartile	Maximum
bFGF								
Baseline	47	2.50	3.10	1.00	1.00	1.27	2.58	16.78
Diff-2mth	30	-0.53	3.18	-15.78	-0.38	0.00	0.66	2.81
%-2mth	30	21.70	78.42	-94.04	-25.93	0.00	66.00	281.00
Last	47	3.45	6.57	1.00	1.00	1.64	2.95	44.07
Diff-last	47	0.95	7.03	-15.54	-0.35	0.00	0.69	43.07
%-last	47	140.49	638.26	-92.61	-25.93	0.00	69.00	4307.00
VEGF								
Baseline	47	290.86	199.18	31.20	159.39	268.95	378.22	982.59
Diff-2mth	30	-92.57	141.24	-536.78	-168.46	-98.812	0.00	118.04
%-2mth	30	-15.86	59.19	-86.70	-51.18	-32.98	0.00	217.18
Last	47	261.57	197.70	31.20	98.96	234.77	409.45	834.13
Diff-last	47	-29.30	161.25	-463.91	-117.68	-4.63	43.87	556.89
%-last	47	2.94	70.65	-87.55	-42.37	-12.27	22.07	233.43
TSP-1								
Baseline	47	3758.12	4653.76	100.00	691.90	2065.70	4925.10	17764.20
Diff-2mth	30	-2394.65	5083.98	-15634.00	-4369.30	-200.55	313.60	5106.20
%-2mth	30	0.65	101.70	-99.31	-84.15	-13.69	44.79	291.40
Last	47	4132.81	6644.36	100.00	100.00	1607.30	6509.10	31838.40
Diff-last	47	374.69	5905.19	-16367.40	-826.10	0.00	635.40	17373.10
%-last	47	364.62	2302.75	-99.39	-51.63	0.00	64.04	15776.20
Endostatin								
Baseline	47	144.23	145.16	4.00	49.01	87.21	197.36	572.54
Diff-2mth	30	9.28	142.31	-342.19	-19.85	7.27	39.35	362.16
%-2mth	30	391.73	1671.95	-98.50	-31.04	9.43	32.79	9054.00
Last	47	132.59	133.32	4.00	44.54	82.54	187.3	598.51
Diff-last	47	-11.64	133.59	-448.55	-50.57	0.00	29.17	362.16
%-last	47	349.67	1463.81	-99.12	-29.65	0.00	40.24	9054.00

Abbreviations: bFGF, basic fibroblast growth factor; VEGF, vascular endothelial growth factor; TSP-1, thrombospondin-1.

^aBaseline = baseline values – first time point; diff = difference between 2-month (diff-2mth) or last (diff-last) serum level and baseline serum level; % = percent change at 2-month (%-2mth) or last (%-last) value from baseline = (diff/baseline serum) \times 100; last = last values – last time point.

high-grade gliomas showed that the drug is well tolerated.^{16,17} In the study by Fine et al.,¹⁶ of the 36 evaluable patients, 4 had objective radiographic regressions (on MRI scans) lasting between 2 and 9 months, and another 12 patients had stabilization of disease for at least 2 months. In this study, a statistically significant relationship between changes in serum bFGF levels and radiographic response and survival was observed. Specifically, patients with stable bFGF survived longer than those with increasing bFGF. In the study by Marx et al.,¹⁷ 42 patients with recurrent glioblastomas were treated with thalidomide (100–500 mg/day; median, 300 mg/day). The regimen was fairly well tolerated: of 38 patients with evaluable disease, two (5%) achieved partial response and 16 (42%) had stable disease. The median survival was 31 weeks, and the 1-year survival was 35%. A phase II study of thalidomide and carmustine in recurrent high-grade gliomas showed a higher overall response rate of 48% compared with historical controls of 20%–30% for carmustine alone and a 24% objective radiographic response rate,¹⁸ suggesting that the combination of thalidomide with a chemotherapeutic agent may result in increased antitumor effects.

Temozolomide has been combined with thalidomide

in several studies of newly diagnosed glioblastomas. Chang et al.³⁹ treated 67 glioblastoma patients with radiation therapy (60 Gy delivered in 2-Gy fractions over 6 weeks) concurrently with temozolomide (150 mg/m²/day for 5 consecutive days for the first 28 days, escalated to a maximum dose of 200 mg/m²/day for 5 days every 28 days) and thalidomide (up to 1,200 mg/day). In their study, the median PFS was 22 weeks (5.5 months) and the median OS was 73 weeks (18.3 months). The regimen was relatively well tolerated and the results appeared to be superior to those for a historical control group treated with radiation alone. However, the benefit of the addition of thalidomide to temozolomide was unclear. Baumann et al.⁴⁰ treated 44 patients with glioblastoma in the postradiation setting with temozolomide and thalidomide. Median PFS was 36 versus 17 weeks (9 vs. 4.3 months) ($p < 0.06$), with a median OS of 103 weeks (25.8 months) for patients receiving both drugs versus 63 weeks (15.8 months) for those receiving thalidomide alone ($p < 0.01$). This suggested that the addition of temozolomide to thalidomide might improve efficacy, although the study included only relatively small patient numbers in each group. In addition, only patients who received thalidomide for 3 months were

Table 3. Adverse events related to treatment regimen (*n* = 50)

	Grade 3	Grade 4/5
Agitation	2	0
Colitis	0	1
Constipation	6	0
Dizziness	1	0
Dyspnea	1	0
Fatigue	2	1
Hypotension	1	0
Infection	1	1
Irregular menses	1	0
Leukopenia	8	2
Lymphopenia	2	0
Memory loss	1	0
Nausea/vomiting	1	0
Neutropenia	9	3 (1 grade 5) ^a
Ototoxicity	1	0
Rash	1	0
Seizure	2	0
Somnolence	0	1
Thrombocytopenia	2	3
Thrombosis	2	2
Tremor	1	0
Vision (blurred)	1	0

^aPatient with neutropenia and fever of unknown origin.

evaluated, introducing an important selection bias. In our study, from the date of diagnosis the median PFS was 9.3 months (95% CI: 8.2–10.9) and the median OS was 16.1 months (95% CI: 11.8–19.3). These results are comparable to those of Chang et al.³⁹ and inferior to those of Baumann et al.⁴⁰ using temozolomide with thalidomide in newly diagnosed glioblastomas. In the study by Chang et al., temozolomide and thalidomide were administered with radiation therapy, potentially contributing to their improved results.³⁹ Although there are important differences in the design of these various studies, our study suggests that it is unlikely that the addition of thalidomide and celecoxib to temozolomide provided a significant survival advantage.

Our study had several limitations. The study was initiated prior to the publication of the European Organization for Research and Treatment of Cancer (EORTC)/National Cancer Institute of Canada (NCIC) study showing a benefit of radiotherapy with concomitant and adjuvant temozolomide in newly diagnosed glioblastoma.¹ As a result, temozolomide was administered only in the adjuvant setting and not concomitantly with radiotherapy. Whether administration of temozolomide with thalidomide and celecoxib with radiation therapy would have improved the results is unclear. The improved survival in the study by Chang et al.³⁹ may be due to the concurrent use of temozolomide and thalidomide with radiotherapy. A second potential limitation is that patients were enrolled after radiotherapy and had to have stable disease. As a consequence, patients who had disease progression during radiotherapy were excluded. This resulted in the enrollment of a group of patients with

a relatively good prognosis and prevented direct comparison of this study's results with those of the EORTC/NCIC study or the majority of the Radiation Therapy Oncology Group trials, in which patients were enrolled prior to radiotherapy. Because of these limitations, we used for comparison the study of Schold et al.,³⁷ which evaluated AZQ and carmustine in patients with newly diagnosed malignant gliomas and which had a design similar to that of our study. Although the 4-month PFS was slightly higher (63% vs. 50%) in this study than in that of Schold et al., the difference did not reach statistical significance. The EORTC/NCIC trial reported by Stupp et al.¹ had a median OS of 14.6 months, comparable to the 16.1 months in this study, with the above caveat that we included a favorably biased population compared with the Stupp et al. study. A third potential limitation is the dose of celecoxib selected for the study. This dose of 400 mg twice daily is higher than the standard therapeutic dose for arthritis and equivalent to the dose used for some of the chemoprevention studies in colorectal cancer.⁴¹ However, a recent study by Reckamp et al.⁴² suggested that the optimal dosing of celecoxib for lung cancer, which resulted in synergism with erlotinib, was 600 mg given twice daily. Thus, we may not have used the optimal dose of celecoxib in our study.

One mechanism by which antiangiogenic therapy may mediate its effects is via increasing TSP-1 levels in animals.⁴³ We sought to identify such serum biomarkers of antiangiogenic activity, but we did not find any of the four tested markers (VEGF, bFGF, endostatin, and TSP-1) to be of prognostic significance, except for a trend in VEGF levels being associated with poorer survival and PFS. This may in part be related to the low response rate and the small sample size. However, we did not measure circulating endothelial progenitor cells, which were reported to be decreased by metronomic chemotherapy in preclinical studies.⁴⁴ Evaluation of circulating endothelial progenitor cells and circulating endothelial cells may be of interest in future studies using such approaches. Unfortunately, due to the inability to retrieve high-quality DNA from archival specimens, we were unable to correlate response with MGMT status. When possible, frozen tissue should always be prospectively saved for such molecular analysis.

In conclusion, we have reported the first use of the combination of temozolomide with the antiangiogenic agents thalidomide and celecoxib in adult patients with newly diagnosed glioblastoma. Although the regimen was moderately well tolerated, it did not appear to significantly increase survival. A number of other studies evaluating this regimen are in progress. However, with the recent availability of potent inhibitors of VEGF and VEGF receptor,^{45,46} it is likely that the combination of temozolomide with such inhibitors will have a greater chance of improving outcome in patients with newly diagnosed glioblastomas than the agents used in this study.

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